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Abstract

Tumor-associated macrophages are a major constituent of malignant tumors and known to stimulate key steps in tumor progression. In our review in this journal in 2006, we postulated that functionally distinct subsets of these cells exist in different areas within solid tumors. Here, we review the many experimental and clinical studies conducted since then to investigate the function(s), regulation and clinical significance of macrophages in these sites. The latter include three sites of cancer cell invasion, tumor nests, the tumor stroma, and areas close to, or distant from, the tumor vasculature. A more complete understanding of macrophage diversity in tumors could lead to the development of more selective therapies to restore the formidable, anti-cancer functions of these cells.

Introduction

Tumor-associated macrophages (TAMs) are abundant in most types of malignant tumor and promote tumor angiogenesis, the escape of cancer cells from the tumor into the circulation, and the suppression of anti-tumor immune mechanisms. They also help circulating cancer cells to extravasate at distant sites like the lungs and then promote their survival and persistent growth into metastatic colonies. An increasing number of studies have also shown that TAMs can either antagonize, augment or mediate the antitumor effects of cytotoxic agents, tumor irradiation, anti-angiogenic/vascular damaging agents, and checkpoint inhibitors (1-3).

The origin(s) of these cells is currently a topic for debate. Recent studies have shown that macrophages in many steady-state tissues are not derived from circulating monocytes as originally thought, but rather from embryonic macrophages (particularly from the yolk sac) that are laid down in tissues during development. These progenitors persist into adulthood by local proliferation, and thus maintain themselves independently of the adult hematopoietic system. Alternatively, in some adult tissues like the intestines, macrophages derive form the bone marrow via monocyte recruitment (4). Initially, TAMs in mouse tumors were also thought to be derived largely from bood monocytes (5), however, recent studies have shown that, in some mouse models of brain and pancreatic cancer, they are derived from *both* blood monocytes and the embryonic macrophages. Moreover, the selective depletion of each of these two TAM subtypes showed that only the latter supported the growth of established tumors (6,7). Further studies are now underway to see if this mixed ontogeny extends to other tumor types.

TAMs often exhibit an array of activation states. In general, they are skewed away from the 'classically' activated, tumoricidal phenotype (sometimes referred to as M1) towards an 'alternatively' activated tumor-promoting one (M2). However, like macrophages in many other tissues, TAMs show remarkable functional plasticity and often express markers characteristic of both activation states (1,8) making such binary definitions inaccurate. In our review In Cancer Research in 2006 (9), we proposed that TAM functions might, at least in part, be regulated by their location

within tumors. We suggested that they exhibited markedly different functions in least three tumor sites; areas of invasion by cancer cells in early tumor development, the stroma, and hypoxic/necrotic areas. Since then, a considerable number of studies have investigated their functions and regulation in these - and other - sites in mouse tumor models, and examined the clinical significance of these spatially distinct TAM subsets (**Table**). So, we now outline the progress made in understanding TAM behavior in the following tumor sites: three areas of cancer cell invasion; areas of high cancer cell density (the so called tumor 'nests'); the perivascular niche close to tumor blood vessels; and poorly vascularized, hypoxic/necrotic tumor areas (**Figure**). We also discuss the clinical/therapeutic implications of these TAM subsets.

Invasive Areas

There are at least three main sites where the increased invasive behavior of cancer cells has been detected during tumor progression. First, around pre-invasive lesions where the uncontrolled proliferation of newly transformed, neoplastic cells leads to their invasion through the basement membrane into the surrounding normal parenchyma to form a carcinoma. This has been well described in tissues like the mammary gland where cancer cells invade through the duct or lobule wall to become an invasive carcinoma. Then, in established tumors - at the 'tumor-stroma border (TSB)' between cancer cell nests and the stroma within the tumor mass, and at the 'invasive front (IF)' where cancer cells invade into surrounding normal tissues (**Figure**).

In our review in 2006 (9), we described the early evidence for macrophages gathering around ducts in adenomas in the mammary glands of MMTV-PyMT mice and promoting their transition to invasive lesions. At the time, this had been demonstrated by crossing MMTV-PyMT mice with a strain carrying a recessive null mutation in the gene encoding colony stimulating factor (CSF-1). The resultant macrophage depletion delayed the progression of pre-invasive lesions into invasive, metastatic carcinomas (10). Other studies had suggested that macrophages might promote invasion of newly transformed cancer cells in pre-invasive mammary lesions by releasing the enzymes,

cathepsins and matrix metalloproteinases (MMPs), as well as the cytokines, epidermal growth factor (EGF) and tumor necrosis factor alpha (TNFα). These were thought to then remodel the extracellular matrix, promote disruption of the basement membrane, accelerate the motility of cancer cells, and increase the migration of cancer cells. More recently, a number of experimental studies have confirmed the important role of macrophages in the transition of pre-invasive, hyperplastic mammary lesions to early invasive carcinoma. For example, in MMTV-iFGFR1 mice, progression failed to occur when macrophages were depleted in mice bearing hyperplastic lesions (11). Macrophages were also shown to stimulate the progression of pre-invasive lesions in a transplantable, p53-null model of early mammary cancer (12). We also showed that the release of vascular endothelial growth factor A (VEGFA) by macrophages around pre-neoplastic lesions in MMTV-PyMT mice to be essential for the 'angiogenic switch' that occurs when these lesions progress to early carcinomas (13,14). Another study showed that macrophages around such preinvasive mammary lesions in mice release CXCR2-binding chemokines, CXCL1 and CXCL5, which promote the migration and invasion of neighboring pre-neoplastic epithelial cells. Here, a subset of macrophages expressing the cell surface proteins, mannose receptor C type 1 (MRC1 or CD206), class A macrophage scavenger receptor (CD204) and major histocompatibility complex II (MHCII), were recruited to ductal hyperplastic lesions. When these were depleted using clodronate liposomes, their progression to invasive tumors was markedly delayed (15) (**Figure**).

Finally, a recent study in a $KRas^{G12D}$ model of lung cancer has shown that deregulated oncogenes in cancer cells like Myc trigger the transition of indolent lung adenomas to aggressive adenocarcinomas. This is because changes in Myc stimulated an increase in CCL9 and IL-23 expression by lung epithelial cells. CCL9 then stimulated the accumulation of $VEGFA^{+}$ macrophages (and thus tumor angiogenesis), and their PD-L1-dependent expulsion of T and B cells. Additionally, IL-23 prompted the exclusion of adaptive T and B cells and cytotoxic NK cells (16).

These findings in mice are supported by clinical studies comparing macrophage levels in low- versus high-grade human ductal invasive in situ carcinomas (DCIS). These lesions are thought to

develop into invasive carcinomas of the breast. High-grade DCIS lesions (especially those filled with cancer cells and containing a central area of necrosis, namely 'comedo DCIS') are more aggressive and have a greater tendency to become invasive than low-grade DCIS. Higher numbers of $CD68^+$ macrophages have been reported in and around high-grade comedo DCIS than low-grade ones (17). Moreover, analysis of gene expression in 40 cases of DCIS showed that genes upregulated by macrophages following their exposure to a key stimulus upregulated in tumors, CSF-1 were more prevalent in high-grade than low-grade lesions (18).

A number of intravital imaging studies have demonstrated the abundance and characteristics of TAMs in the TSBs of MMTV-PyMT tumors. At least two TAM subsets were present: motile, $MRC1^-$ and relatively immobile, $MRC1^+$ (19,20). Interestingly, high numbers of $CD68^+$ TAMs in the TSBs of human colon carcinomas correlate with better overall survival than those with lower numbers (21) (**Table**). However, their MRC1 status was not investigated in this clinical study - they were just labelled with an antibody for the pan macrophage marker, CD68 – so it remains to be seen whether these two subsets were present, and if one or both contributed to the improved prognosis.

It is noteworthy that antibodies against CD68 continue to be used widely to immunolabel TAMs in such human tumors (**Table**). However, as with many antibodies supposedly labelling individual cell types, those for human CD68 sometimes label cells other than TAMs. For example, a qualitative, immunostaining study reported that some CD68+ cells in human breast tumors fail to express detectable CSF1R or CD45, or markers for epithelial cells, endothelial cells, or mural cells (ie. vascular smooth muscle cells, pericytes or fibroblasts) (22). The identity of these CD68+ cells - and whether they exist in other tumor types – has yet to be elucidated.

When it comes to the IF of tumors, TAMs in these regions of mouse RIP1-Tag2 pancreatic tumors have been shown to enhance the invasive potential of cancer cells via their expression of cathepsins B and S, two enzymes regulated by IL-4 released by cancer cells and tumor-infiltrating T cells (23). Further, CD4+T cells in MMTV-PyMT tumors have been shown to increase the invasiveness of cancer cells via their release of IL-4 which then stimulates TAMs to express EGF release (24).

Together, these experimental data accord well with a previous finding showing that TAMs in the IF of human gastric tumors express the matrix-degrading enzyme, MMP 9, and the receptor for the serine protease, urokinase-type plasminogen activator (uPA; which cleaves pro-UPA into its active form) (25). Interestingly, TAMs along the IF of primary human colon carcinomas express CD80 and CD86 (costimulatory signals necessary for T cell activation), suggesting that they may have the potential to help stimulate anti-tumor immunity in this type of cancer (26). This could explain the observation that high $CD68^+$ TAM levels in the IF of human colorectal tumors correlate with a higher relapse-free survival (RFS) (27) (**Table**). However, various TAM subsets may be present in the IF of tumors with some appearing to be immunosuppressive. For example, TAMs in the IF of human hepatocellular carcinomas (HCCs) express higher levels of the immunosuppressive, negative checkpoint regulator, PD-L1, than those in neighboring cancer nests, and have been linked to poor survival (28). Furthermore, semaphorin 4D (SEMA4D, CD100), a cytokine upregulated in the IF of Colon26 mouse colon tumors, has been shown to stimulate the number of TAMs expressing the immunosuppressive cytokine interleukin 10 (IL-10) in the IF, and thus suppress the number of activated $CD8^+$ T cells in this location. Antibody blockade of SEMA4D suppressed the number of these TAMs at the IF and increased the treatment efficacy of checkpoint inhibitors anti-PD-1 and anti-CTLA4 (29) (**Figure**).

Cancer Nests

The possible function(s) of TAMs in close proximity to cancer cells in tumor 'nests' appears to vary with tumor type. For example, TAMs expressing NOS2, an enzyme linked to the cytotoxic potential of TAMs (via its production of nitric oxide), are seen in intimate contact with cancer cells in some human prostate tumors (30), and high numbers of nest TAMs correlate with an improved prognosis in endometrial cancer (31), and a reduced recurrence in gastric cancer (32) (**Table**). However, high nest TAMs also correlate with reduced overall and RFS in malignant melanomas, as well as breast and esophageal tumors (33-36) (**Table**). TAMs in the nests of human HCCs preferentially express IL-

10 and recruit immunosuppressive FoxP3$^+$ T_{reg} cells (28), although their number has yet to be shown to be associated with outcome in this disease (**Figure**).

Stroma

In this prominent area of most solid tumors, cancer cells are often sparse or absent. Rather, it consists of a complex network of macromolecules in the extracellular matrix (ECM) including collagen fibrils, laminin, fibronectin, tenascin C and hyaluronic acid (HA). It is often populated by various non-malignant cell populations including fibroblasts, endothelial cells, pericytes, lymphocytes and myeloid cells (37). A number of studies have shown that ECM components (and/or their proteolytic products), such as fibronectin, laminin-10, versican (a chondroitin sulfate proteoglycan), and HA fragments, regulate the phenotype of macrophages (38). Moreover, Pinto and colleagues (39) showed recently that decellularized ECM isolated from human colorectal tumors stimulates macrophages to express a relatively anti-inflammatory, M2-like phenotype with increased expression of IL-10, transforming growth factor β (TGF-β), chemokine (C-C motif) ligand 18 (CCL18), and decreased C-C chemokine receptor type 7 (CCR7), TNFα and interleukin-6 (IL-6) *in vitro*. This agrees with a number of studies showing a correlation between high numbers of stromal TAMs in breast, esophageal, gastric, pancreatic, oral and skin tumors and poor overall survival and/or RFS (34,35,40-44) (**Table**). However, this may depend on tumor type as there is no such correlation in endometrial, cervical, and lung cancer (30,45,46), and in bladder cancer, it even correlates with reduced lymph node metastasis and improved survival (47) (**Table**).

In addition to the effects of a complex array of components in the 'matrisome' of the stroma (i.e. the core ECM proteins including collagens, fibronectins, laminins, proteoglycans, growth factors, chemokines and cytokines, and ECM-remodeling enzymes), the biophysical properties of the stroma also regulate the functions of TAMs. The architecture and stiffness of the ECM have been shown previously to regulate cell behavior (38), and increased substrate stiffness upregulates the expression of various pro-inflammatory genes by macrophages *in vitro* by activating TLR4 signaling

pathways in these cells (48). Possible effects of matrix rigidity on macrophages in the premetastatic niche have also been reported as the cross-linking of collagens and elastins induced by the enzyme, lysyl oxidase (LOX), modifies the recruitment, invasion and retention of myeloid cells (49). In an interesting, recent study, high levels of 22 common matrisome constituents (termed the 'matrix score') positively correlated with both tumor stiffness and TAM infiltration in ovarian metastases, although it remains to be seen whether the last two are causally linked (50). To add to this complex picture, it should be noted that different areas of stroma within a given tumor may differ in their chemical and biophysical properties and so regulate TAMs differently (**Figure**).

Interestingly, macrophages in some tissues appear to play an important role on collagen remodelling. Proteolyzed fibrillar collagen recruits macrophages during postpartum mammary involution in rats (51) and macrophages have been shown to facilitate collagen fibrillogenesis in developing mammary glands in mice (52). Given that fibrillar collagen is abundant in stroma of tumors, studies are now warranted to see if this two-way interaction occurs there, and what effects this has, if any, on tumor progression and response to various treatments.

Perivascular Niche

A subset of TAMs lie close to, or on, the abluminal surface of blood vessels in mouse and human tumors (53). These perivascular (PV) cells often express high levels of the M2-associated markers, TIE2 (a major receptor for angiopoietins), MRC1 and CD163, and play a key role in stimulating tumor angiogenesis, metastasis and relapse after frontline treatments for cancer (9). Due to their relatively high expression of TIE2, these cells were initially termed 'TIE2-expressing monocytes/macrophages (TEMs)'. When co-injected into mice with mouse mammary cancer cells, the resultant tumors were more vascularized than generated with cancer cells alone or cancer cells with $TIE2^{-}$ monocytes (54). Interestingly, the frequency of TEMs has also been shown to positively correlate with MVD in some human tumor types (55,56) (**Table**).

Genetic deletion of PV TIE2$^+$ TAMs or the pharmacological blockade of the main TIE2 ligand upregulated by the tumor vasculature, angiopoietin 2 (AGPT2), demonstrated the importance of this TAM subset in tumor angiogenesis and growth in various mouse models of cancer (57). The subsequent gene expression profiling of TEMs isolated from mouse tumors revealed their higher expression of a number of tumor-promoting genes including *Mmp9, Vegfa, Cxcl12, Tlr4* and *Nrp1*, than TIE2$^-$ TAMs from the same tumors (58).

Intravital imaging studies have shown that some PV TIE2$^+$VEGFA$^+$ TAMs interact closely with both endothelial cells and cancer cells expressing actin binding protein mammalian enabled (MENA). These cell trios have been termed the 'tumor microenvironment of metastasis' (TMEM) as they are sites of increased intravasation of cancer cells into the blood. PV TAMs in TMEMs upregulate VEGFA and increase the permeability of neighboring blood vessels (59). Their role in promoting metastasis is supported by the finding that high TMEM frequency correlates with increased risk of distant metastasis in ER$^+$HER2$^-$ breast cancer patients (60). Interestingly, a recent study has shown that TMEMs containing TIE2$^+$VEGFA$^+$ PV TAMs are also present in pre-malignant lesions in a mouse model of HER2$^+$ breast cancer, and promote the early dissemination of cancer cells (61) (**Figure**).

PV TIE2$^+$ TAMs have also been implicated in the relapse of primary mouse tumors after various forms of treatment. They increase in relapsing glioma after local irradiation, and in lung and mammary tumors after chemotherapy. At such times, they express high levels of CXCR4 and are recruited by upregulated CXCL12 in the perivascular niche (62,63). Our studies showed that this TAM subset then stimulates revascularization and regrowth of tumor via their release of VEGFA (63). A later study confirmed that TIE2 expression at TAMs is required to induce vascularization after chemotherapy in mice (64). Interestingly, CXCL12 released by cancer-associated fibroblasts (CAFs) has now been shown recently to recruit CXCR4$^+$ TAMs into the PV niche even in the absence of such treatments (65).

Finally, in metastatic sites like the lungs, a subset of CCR2$^+$Ly6C/Gr1$^+$ macrophages promote the extravasation of cancer cells and their formation of metastases (5). These 'metastasis-associated

macrophages (MAMs)' have been shown in mouse tumor models to directly tether vascular cell adhesion molecule-1 (VCAM-1) on cancer cells via their α4-integrins, a process that subsequently increases cancer cell survival at such metastatic sites (66) . Furthermore, binding of CCL2 to CCR2 on MAMs stimulates their release of CCL3, which binds to CCR1 on cancer cells and facilitates their retention in the lungs (67). These MAMs also promote persistent growth of metastatic lesions through VEGFR1 and CSFR1 signaling (68,69).

Hypoxic/Necrotic Areas

Hypoxia is a hallmark feature in solid tumors and has been linked to increased invasion and metastasis, resistance to therapy, and poor clinical outcome. Hypoxic areas typically have oxygen tensions (pO_2 values) below 10 mm Hg and are located more than 150 μm from tumor blood vessels. They form in tumors when the cellular requirement for oxygen outstrips its supply by the poorly organized tumor vasculature. These sites have been identified in tumor sections using hypoxic cell markers, e.g. pimonidazole (PIMO), or immunolabelling for the hypoxia-inducible alpha subunit of the transcription factors, HIFs 1 and 2 (70). High numbers of hypoxia TAMs associate with elevated levels of tumor angiogenesis, metastasis, poor RFS and/or reduced overall survival in breast, endometrial and cervical cancer (30,71,72) (**Table & Figure**).

When TAMs gather in such areas they upregulate HIFs 1 and 2, and various HIF target genes like VEGFA, GLUT1 and MMP7 (73,74). TAMs are recruited into these sites by chemokines upregulated due to hypoxia, including C-X-C motif chemokine 12 (CXCL12), endothelial cell monocyte-activating polypeptide-II (EMAP-II), endothelin 2, VEGFA and SEMA3A (75-77). Hypoxic TAMs become immobilized in hypoxic areas by the direct, inhibitory effect of hypoxia on their mobility (78) and their reduced expression of receptors for tumor-derived chemokines CCR2, CCR5 and NRP1 (76).

Hypoxic TAMs promote tumor angiogenesis, immune evasion and metastasis in various experimental models. For example, they upregulate an array of proangiogenic and

immunosuppressive cytokines in hypoxic tumor areas (73,74,76,79), and when their entry into hypoxic tumor areas is impeded by SEMA3A/NRP1 signaling blockade, tumor angiogenesis is markedly reduced, and antitumor immunity restored (77). Hypoxic TAMs are also able to suppress T cell activation in a number of ways including their upregulation of IL-10 and negative checkpoint regulators such as PD-L1 (77). A recent study also showed that macrophages co-cultured with hepatoma cells under hypoxic conditions have increased indoleamine 2, 3-dioxygenase (IDO) expression which suppresses the proliferation of local cytotoxic T cells and expands T_{reg} cells (80).

While exposure to hypoxia *per se* fails to skew TAMs towards a tumor-promoting, phenotype (74,81), some studies have shown that a low pH and lactate (which accumulate in poorly vascularized, hypoxic areas due to the poor vascular supply) act in concert to induce a proangiogenic phenotype in TAMs, which, in turn, restores blood perfusion (81-83). Indeed, lactic acid can stimulate expression of VEGFA by macrophages (83). As mentioned previously, this cytokine is not only proangiogenic in tumors but also capable of stimulating the intravasation of cancer cells. It remains to be seen whether VEGFA released by TAMs in poorly vascularized areas (i.e. away from blood vessels) contributes to the latter phenomenon.

Tumor hypoxia can also modulate TAM functions indirectly by stimulating cancer cells to release high-mobility group box 1 protein (HMGB1) which, in turn, stimulates IL-10 production by TAMs. Furthermore, this hypoxia-HMGB1-IL-10 axis has been shown to stimulate metastasis in the murine B16 tumor model (84). Hypoxia also induces metabolic changes in TAMs which then impact directly on the functions of neighboring cells. For example, hypoxia stimulates their expression of REDD1, an mTOR inhibitor and key modulator of metabolism in response to nutrient availability and energy requirement. The resultant inhibition of mTOR in TAMs strongly reduces their glucose uptake and glycolysis, leaving more glucose for neighboring endothelial cells. This results in a more hyperactive and leaky vascular network and the provision of more escape sites for cancer cells into the circulation. So, it is hardly surprising that this mechanism in primary tumors has been shown to drive the formation of distant metastases (85).

Concluding Remarks

A number of experimental studies in mice have now confirmed the ability of different tumor compartments to differentially regulate the phenotype of TAMs. The importance of this is underscored by clinical reports showing that the number and/or phenotype of TAM in specific tumor areas correlate with RFS and/or survival in human tumors (**Table**).

We are beginning to identify the factors regulating this spatial heterogeneity of TAMs in tumors. As mentioned earlier, genetic changes taking place during early neoplasia can be 'sensed' by neighboring macrophages and trigger their tumor-promoting functions. Activation of the oncogene, c-Myc, and mutations in the tumor suppressor gene, P53, in breast epithelial cells are prominent in high-grade DCIS, and regulate the function of macrophages in such in such preinvasive lesions (15,86-88). Later, in established tumors, nest TAMs are exposed to tumor cell-derived factors, hypoxia, low pH and high lactate concentration (due to the tumor vasculature being unable support the metabolic needs of rapidly proliferating tumor cells) (89,90). Alternatively, TAMs in the stroma receive a diverse array of signals, including those released by, or expressed on the surface, of endothelial cells, pericytes, fibroblasts, lymphocytes, other myeloid cells, and ECM constituents. However, it may be over-simplistic to assume that any two similar areas within a tumor (e.g. stromal areas) are identical, and thus regulate TAM behavior in the same way. Furthermore, the phenotype of TAMs in a given area will likely change over time as each site changes within the tumor mass.

It also remains to be seen whether TAMs in different tumor areas are variants of the same TAM pool conditioned to perform specific functions in response to local signals, or whether they also have different origins. For example, in some tumor areas, TAMs may be recruited from circulating monocytes (or distinct monocyte subsets) as they are in the PV niche (65), while in others they may derive from the proliferation of a local TAM pool (91). As mentioned earlier, a recent cell fate-mapping study has shown that TAMs in mouse brain tumors are derived from *both* resident brain macrophages (microglia) and blood monocytes. While they shared a common, tumor-induced gene

expression signature, they also exhibited considerable differences in their transcriptional profile, suggesting the retention of certain ontogeny-specific characteristics (6,92). However, it is not known yet whether this this phenomenon is restricted to brain tumors, or whether it contributes to the spatial diversity of TAMs in tumors. We also have much to learn about the development of TAM subsets in different areas of *metastatic* tumors, as virtually every study on this so far has been in the primary setting.

Evidence is also emerging for the role of TAM subsets in certain tumor areas limiting tumor responses to treatment. For example, irradiation, vascular disrupting agents, and cytotoxic drugs induce the expansion of the perivascular TAMs, which, contributes to tumor angiogenesis and relapse after therapy (66,67,93). Hypoxic TAMs have also been implicated in tumor resistance to several anticancer treatments and to promote relapse (94).

As TAMs have been shown to stimulate so many tumor-promoting mechanisms, the development of therapeutic approaches to deplete or reprogram them is a rapidly emerging field. For example, a variety of inhibitors for CSF-1 or CSF-1R (CD115), have shown efficacy in depleting TAMs in pre-mouse tumor models and are being tested currently in clinical trials. However, their efficacy in depleting monocytes and macrophages throughout the body has led to potentially limiting side effects like periorbital edema (95-97). Advances in our understanding of how the phenotype of TAM subsets in different tumor areas is influenced by their ontogeny, activation status and complex array of local cues will help to develop new, more selective therapeutic approaches. Unravelling such a complex array of influences on TAM behavior may require a multifaceted approach including cell fate mapping studies, high-dimensional, single-cell analysis techniques, and systems biology/computer modelling. However, this could then lead to more effective, personalized approaches for the selective targeting of appropriate TAM subsets.

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Figure & Table Legends

Figure. The phenotype of TAMs in different compartments within established primary tumors. A small sub-compartment within a tumor is shown consisting of 3 tumor 'nests' (areas of high cancer cell density) containing hypoxic/necrotic (H/N) areas; the tumor-stroma border (TSB) at the edge of tumor nests (grey dashed line); the stroma (which in most solid tumors is highly vascularized; red); and an invasive front (IF) between the tumor mass and surrounding non-malignant tissue (blue dashed line). Box – cell surface markers, enzymes and cytokines expressed by TAMs in these different regions.

Table. TAMs in different areas of human tumors: correlation with important clinico-pathological features. CD68 used to immunolabel TAMs in tumor sections unless otherwise stated. [Abbreviations used: ND, not determined; IF, invasive front of tumor; MVD, microvessel density; PV, perivascular; OS, overall survival; RFS, relapse free survival; PFS, progression free survival; LNM, lymph node metastases; TMEM, tumor microenvironment of metastasis].